# DYSREGULATION OF $Ca^{2+}$ HOMEOSTASIS CONTRIBUTES TO EXTRACELLULAR α-SYNUCLEIN-MEDIATED TOXICITY

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α-Synuclein (AS) is an abundant presynaptic neuronal protein genetically and biochemically linked to Parkinson's disease (PD) pathogenesis. Data from our laboratory and others suggest that AS can be normally secreted from neuronal cells. To assess the effects of secreted AS on neuronal homeostasis, we have used SH-SY5Y cells inducibly expressing human wild-type AS**,** as a source of naturally secreted AS. We have shown that these cells readily secrete AS species into their culture medium, partly via an exosome-mediated manner. Secreted AS forms were shown to be toxic to recipient neuronal cells. To investigate the possible mechanisms involved in the extracellular AS-mediated toxicity, we applied naturally secreted AS to differentiated SH-SY5Y cells. Our results show that such treatment alters $Ca^{2+}$ homeostasis in recipient neuronal cells, manifested by increased capacitative $Ca^{2+}$ entry, i.e. $Ca^{2+}$ influx upon depletion of intracellular $Ca^{2+}$ stores with thapsigargin (Tg). This effect was AS-dependent, since immunodepletion of the protein from the conditioned medium (CM) abolished $Ca^{2+}$ influx. Importantly, the observed $Ca^{2+}$ rise was attributed mainly to exosome-associated AS, given the fact that cells pretreated with exosomes isolated from AS-containing CM exhibited higher $Ca^{2+}$ influx compared to cells recipient of exosome-depleted CM. Voltage Operated $Ca^{2+}$ Channels (VOCs) were involved in the elevated cytosolic $Ca^{2+}$ rise, since the use of specific inhibitors ameliorated AS-induced $Ca^{2+}$ influx. Differentiated neuroblastoma cells incubated either with VOC blockers or with extracellular or intracellular $Ca^{2+}$ chelators were resistant to secreted AS-mediated toxicity. Collectively, our data suggest that secreted AS, and particularly its exosomal component, is toxic to recipient neuronal cells through engagement, at least partly, of the intracellular $Ca^{2+}$ homeostatic machinery. Manipulating $Ca^{2+}$ signaling pathways mitigates extracellular AS toxicity and may therefore represent a potential therapeutic target for PD and related synucleinopathies.

**8th Forum of European Neuroscience (FENS), 14-18 July 2012, Barcelona, Spain**

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MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS, CULTURE & SPORTS
MANAGING AUTHORITY
NSRF 2007-2013
programme for development
EUROPEAN SOCIAL FUND
Co- financed by Greece and the European Union